FORM PTO-1390 (Modified) (REV 5-93) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 24741-1525

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5): 09/856694

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/09067	24 November 1999	25 November 1998

TITLE OF INVENTION
HYPERFORIN AS A CYTOSTATIC AGENT AND HYPERFORIN OINTMENT OR CREAM AS AN APPLICATION FORM

APPLICANT(S) FOR DO/EO/US
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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. _x_ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ___ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ___ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. _x_ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. _x_ A copy of the International Application as filed (35 U.S.C. 371©(2))
   a. _x_ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ___ has been transmitted by the International Bureau.
   c. ___ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. _x_ A translation of the International Application into English (35 U.S.C. 371 ©(2)).
7. _x_ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371©(3))
   a. ___ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ___ have been transmitted by the International Bureau.
   c. ___ have not been made; however, the time limit for making such amendments has NOT expired.
   d. _x_ have not been made and will not be made.
8. ___ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371©(3)).
9. ___ An oath or declaration of the inventor(s) (35 U.S.C. 371©(4)).
10. ___ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371©(5)).

Items 11. to 16. below concern other document(s) or information included:

11. _x_ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ___ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. _x_ A FIRST preliminary amendment.
    ___ A SECOND or SUBSEQUENT preliminary amendment.
14. ___ A substitute specification.
15. ___ A change of power of attorney and/or address letter.
16. _x_ Other items or information: **Unexecuted Declaration; PTO/SB/08A (including 19 references)**

JC18 Rec'd PCT/PTO 24 MAY 2001

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
-NEW 09/856694	PCT/EP99/09607	24741-1525

17. ☐ The following fees are submitted:

	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5): Neither international preliminary examination fee (CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....**$1000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO . **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....**$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4).....**$690.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)..... ..**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than 20 ___ 30 ___ months from the earliest claimed priority date (37 CFR 1.492(e))	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	20 -20 =	0	X $18.00	$	
Independent Claims	2-3 =	0	X $80.00	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)		0	+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$0.00	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$430.00	
SUBTOTAL =				$430.00	
Processing fee of $130.00 for furnishing English translation later the 20 ___ 30 ___ months from the earliest claimed priority date (37 CFR 1.492(f). +				$	
TOTAL NATIONAL FEE =				$430.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$430.00	
				Amount to be: refunded	$
				charged:	$

a. ___ Our check no. _____ in the amount of $860 to cover the above fees is enclosed.

b. X Please charge my Deposit Account No. 08-1641 in the amount of **$430.00** to the above fees. A duplicate copy of this sheet is enclosed.

c. X The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-1641. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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26633
PATENT TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of: Jan SIMON *et al.*

Serial No.: Unassigned Art Unit: Unassigned

Filed: Concurrently Examiner: Unassigned

For: HYPERFORIN AS A CYTOSTATIC AGENT AND HYPERFORIN OINTMENT OR CREAM AS AN APPLICATION FORM

Preliminary Amendment

Director, United States Patent and Trademark Office

Washington, DC 20231

Dear sir:

Before action by the Examiner, please amend the captioned application as set forth below.

In the Claims:

Please cancel claims 1-19 without prejudice or disclaimer and add the following new claims:

20. **(New)** A pharmaceutical composition comprising:

a) either i) an effective amount of hyperforin, or ii) an effective amount of hyperforin and hypericins; and

b) a carrier acceptable for topical administration.

21. **(New)** The pharmaceutical composition according to claim 20, wherein said effective amount of hyperforin and hypericins is present in the form of an extract.

22. **(New)** The pharmaceutical composition according to claim 21, wherein said extract is an alcoholic extract.

23. **(New)** The pharmaceutical composition according to claim 22, wherein said alcoholic extract comprises at least about 20-60% v/v of ethanol.

24. **(New)** The method according to claim 22, wherein said alcoholic extract comprises at least about 40-50% v/v of ethanol.

25. **(New)** The pharmaceutical composition according to claim 21, wherein said extract comprises St. John's wort extract.

26. **(New)** The pharmaceutical composition according to claim 25, wherein said St. John's wort extract is present in an amount of at least 5% by weight.

27. **(New)** The pharmaceutical composition according to claim 20, wherein the carrier comprises an ointment.

28. **(New)** The pharmaceutical composition according to claim 20, wherein the carrier comprises a cream.

29. **(New)** The pharmaceutical composition according to claim 20, wherein hyperforin is present in a concentration of at least about 15 μg per ml.

30. **(New)** The pharmaceutical composition according to claim 20, wherein hyperforin is present in a concentration of at least about 1-20 mg per ml.

31. **(New)** The pharmaceutical composition according to claim 20, wherein hyperforin is present in a concentration of at least about 0.02-20 mg per ml.

32. **(New)** The pharmaceutical composition according to claim 20, wherein said composition further comprises hypericins in a concentration of at least about 15 μg per ml.

33. **(New)** A method of preparing a pharmaceutical composition comprising the step of mixing:

a) either i) an effective amount of hyperforin, or ii) an effective amount of hyperforin and hypericins; and

b) a carrier acceptable for topical administration.

34. **(New)** The method according to claim 33, wherein said effective amount of hyperforin and hypericins is in the form of an extract.

35. **(New)** The method according to claim 34, wherein said extract comprises St. John's wort extract.

36. **(New)** A method for treating a disease selected from the group consisting of: cancer; inflammatory skin diseases; precancerous conditions; geriatric skin; and microbial skin infections, comprising administering to a skin of a subject in need thereof an effective amount of a composition according to claim 20.

37. **(New)** The method according to claim 36, wherein the disease is ecezema.

38. **(New)** The method according to claim 36, wherein said disease is selected from the group consisting of: exsiccation eczemas; hyperkeratotic hand and foot eczemas; contact eczemas; atopic dermatitis; neurodermatitis; lichen simplex; prurigo simplex; lymphomas; leukemia; melanoma; epithelial pre-cancerous conditions; tumor metastases; and epithelial tumor.

39. **(New)** The method according to claim 36, wherein said subject is a mammal.

Remarks

By the foregoing, claims 20-39 are pending. An Office Action on the merits is now awaited. Should there be any questions, the Examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

Patricia D. Granados

May 24, 2001
Date

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PATENT TRADEMARK OFFICE

24 November 1999
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Hyperforin as a cytostatic agent, and hyperforin ointment or cream as an application form

The present invention relates to the use of hyperforin as a cytostatic agent and to a hyperforin ointment or cream which is suitable as a form for applying the hyperforin.

Hyperforin and the hypericins are characteristic constituents of St. John's wort (Hypericum perforatum L.), which also contains constituents which occur generally in the plant kingdom, such as flavone derivatives, flavonol derivatives, rutin, hyperoside, xanthone derivatives, amentoflavone, biapigenin and ethereal oils.

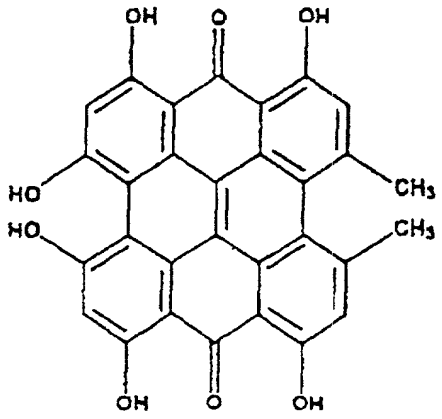
St. John's wort and St. John's wort extracts have already been employed for some considerable time in medicine and folk medicine as drugs for a wide variety of indications. The constituent hypericin has also recently come to be used in drugs as an active compound in isolated form (L. Roth, Hypericum, Hypericin, Ecomed medicinal plant monograph, Ecomed, Landsberg/Lech, 1990).

The monograph "Hyperici herba (St. John's wort)", which was published by commission E of the former Public Health Office on 5.12.1984, specifies the area for using Hypericum preparations (internally as drops or tablets) as being: "psychovegetative disturbances, depressive parathymic conditions, anxiety and/or nervous agitation". The antidepressant activity, which is comparable to that of the tricyclic antidepressants, of St. John's wort has been

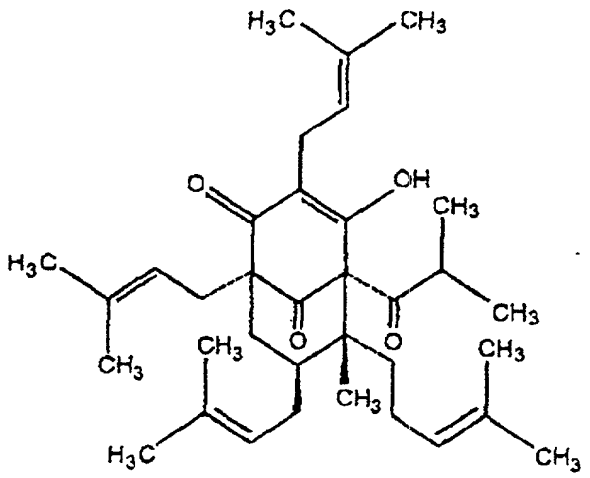
substantiated in a large number of placebo-controlled studies.

As a household remedy, St. John's wort oil is employed, in particular, for treating wounds and pain and in association with burns (L. Roth loc. cit.).

Due to the characteristic red colour and fluorescence of the oil, hypericin was initially assumed to be the active compound in the St. John's wort oil. The formula of hypericin is depicted below:



However, more recent investigations into the composition of St. John's wort oil have shown that it does not in fact contain any hypericin but instead what are termed oil hypericins, which are lipophilic products of the lysis of hypericin. In addition, St. John's wort oil contains hyperforin (P. Maisenbacher et al., Planta Med. 58:351-354 (1992) and B. Hellwig, DAZ 137, 29, pages 35-36), whose formula is depicted below:



As an active compound, hyperforin has aroused interest as an antidepressant substance (Pharmacopsychiatry 1988, Vol. 31, Supplement 1, pages 1-60). In addition to this, hyperforin possesses antibacterial activity (A. I. Gurevich et al., L. Antibiotiki, 16:510-513 (1971)).

Stable extracts prepared from Hypericum perforatum L. have been disclosed in DE 197 14 450 and DE 196 46 977.

DE 195 47 317 discloses an antiviral medicament which is based on St. John's wort active compounds and which comprises 1-50% hypericin or pseudohypericin.

EP-A-0 599 307 discloses a dry extract prepared from St. John's wort having an elevated content of hyperforin and also its use for producing drugs having psychovegetative and antidepressant activity.

Apart from St. John's wort oil, ointments are known which comprise a low concentration of Hypericum in addition to a variety of other medicinal herbs. Examples of these ointments are: "Unguentum Truw" for wound treatment and the homeopathic agent "Traumeel S", which is an antiinflammatory agent, and "Atemaron N R30", which is an analgesic/antirheumatic agent. It is not known whether these ointments contain hyperforin. However, because of the instability of hyperforin, it must be assumed that hyperforin is either not present, or only present in low quantities,

if the extract is not prepared, stored and processed while being protected from light and oxidation. For example, it is not possible to detect any hyperforin in a commercially available Hypericum extract (Hyperforate).

In connection with topical use, and particularly in connection with treating inflammatory skin diseases, the known St. John's wort oil suffers from the disadvantage that, because of its fat content, it can only be used to a limited extent and is not suitable, for example, for treating eczemas. In addition to this, the oil only contains fat-soluble constituents of the St. John's wort and does not contain hypericin, for example.

Cytostatic agents inhibit the growth, in particular, of rapidly growing cells as are found in tumours and leukaemias. They are therefore suitable for use as chemotherapeutic agents for the treatment of cancer. While a large number of chemotherapeutic agents are known, these compounds also suffer from disadvantages. In particular, treatment with known chemotherapeutic agents is accompanied by pronounced side-effects and most chemotherapeutic agents only exhibit satisfactory activity towards particular tumour cell lines.

There therefore continues to be a need for additional compounds which are suitable for treating cancer.

An object of the present invention is therefore to make available an additional chemotherapeutic agent for treating cancer diseases.

Another object of the present invention is to make available a pharmaceutical formulation for the chemotherapeutic agent, which formulation does not suffer from the abovementioned disadvantages. In particular, it should be possible for the formulation to be adapted to a variety of skin conditions.

It has now been found, surprisingly, that hyperforin exerts a proliferation-inhibiting effect on tumour cells and can induce apoptosis in tumour cells.

The present invention consequently relates to the use of hyperforin for producing a drug for treating cancer diseases (primary tumours and metastases) and/or precancerous stages (cancer precursor stages).

In the present instance, cancer diseases are understood, in particular, as meaning malignant tumours and also lymphomas and leukaemias.

However, hyperforin is also particularly active against metastases, such as malignant melanoma (black skin cancer) metastases.

Hyperforin has also proved to be effective against epithelial tumours such as epithelial skin cancer. This cancer is a slowly growing skin cancer which is readily accessible to topical treatment. Epithelial skin cancer is also termed spinalioma, squamous cell carcinoma or prickle cell cancer. In addition to this, hyperforin is also suitable for treating precancerous stages such as solar precancerous stages.

In addition, hyperforin is suitable for treating mammary carcinomas (breast cancer).

In practice, it is of particular interest to use hyperforin in connection with lymphomas/leukaemias and difficultly operable tumours and for the adjuvant treatment of metastases. In particular, only very moderate success has been achieved with the therapies which are so far available for treating malignant melanoma.

Hyperforin can be administered intravenously for treating systemic tumours and metastases. For intravenous administration, the lyophilized or dried active compound can, for example, be dissolved freshly in physiological saline solution and immediately injected or infused. However, the active compound can also be administered orally, for example in tablet form.

However, hyperforin is also suitable for local administration, for example by means of injection or instillation (for example endoscopically as well) within or around the tumour. The active compound can, for example, be prepared for this purpose as described above for the intravenous administration. However, the active compound can also advantageously be applied by epicutaneous application, for example in the form of a cream, with this application form being particularly suitable, for example, for treating solar precancerous stages.

For local, epicutaneous application, the active compound can, for example, be dissolved in ethanol and worked into a greasy ointment base. This can take effect occlusively (under film) on the tumour, for example for a period of 24 hours. Particularly preferred ointments and creams are described in more detail below.

When the active compound is being worked up, attention must be paid to the fact that it is a light-sensitive substance which readily decomposes. Appropriate protection from light must therefore be ensured when the active compound is being isolated, stored and administered.

In the treatment, according to the invention, of cancer diseases and/or precancerous stages with hyperforin, the concentration of hyperforin at the site of action should be sufficiently high to ensure that an antiproliferative or apoptosis-inducing effect is elicited. The concentration which is required for this purpose can vary depending on the nature of the treated tumour and can be readily determined by the skilled person. For example, a hyperforin concentration of 50 µg/ml in the administered solution is advantageous in the case of injection into a tumour while an active compound concentration of 100 µg/µl is advantageous in connection with epicutaneous application. In the case of systemic use, the active compound should be injected in quantities which are sufficient to ensure that

plasma levels of at least 50 µg/ml are achieved. This corresponds to a hyperforin quantity of about 5 mg/kg of patient body weight.

As another aspect of this invention, it has been found that hyperforin can advantageously be administered in a pharmaceutical formulation which is in the form of a topical ointment or cream which comprises at least 15 µg of hyperforin/ml.

The ointment or cream should contain a concentration of active compound which is as high as possible, preferably in the range of 0.02-20 mg of hyperforin/ml, more preferably in the range of 1-20 mg/ml and particularly preferably about 10 mg/ml (1% hyperforin).

In addition to the active compound hyperforin, the ointment or cream according to the invention can additionally comprise hypericins as additional active compounds. In this connection, the total concentration of the hypericins in the ointment or cream should be at least 15 µg/ml, preferably 20-150 µg/ml. In the present instance, hypericins are understood as meaning hypericin and its pharmaceutically active derivatives. These include, in particular, pseudohypericin, which differs from hypericin in that a methyl group is replaced with hydroxymethyl.

The abovementioned active compounds can be introduced into the ointment or cream either as pure substances or in the form of a St. John's wort extract of defined concentration. In this connection, the ointment or cream according to the invention preferably does not comprise any other plant extracts apart from the St. John's wort extract.

For example, a St. John's wort extract which contains at least 200 µg of hyperforin/ml and at least 200 µg of hypericins/ml is suitable for preparing the ointment or cream according to the invention. The extract employed preferably contains 200-100,000 µg/ml, in particular about 1000 µg of hyperforin/ml, and 200-1000 µg of hypericins/ml. With these active

compound concentrations in the extract, the ointment or cream according to the invention should contain at least 5% by weight of the extract. Advantageously, an ointment according to the invention can, for example, contain about 15% by weight of the extract, and a cream according to the invention can contain about 10% by weight of the extract.

The total extract which is standardized for hyperforin and hypericin should be an ethanolic extract or an extract to which ethanol has been added. In this connection, the extracts can, for example, be commercially available total extracts (tinctures). The ethanol content is preferably between 20 and 60% v/v, preferably 40-50% v/v. These requirements are met, for example, by a total extract supplied by the company Caelo, which total extract is preferably employed in accordance with the invention and contains 240 µg of hyperforin/ml and 300 µg of hypericin/ml.

In principle, aqueous extracts, CO_2 extracts or fresh plant extracts are also suitable provided they meet the requirements for the content of active compound.

The St. John's wort extract which is used for the ointment or cream according to the invention is qualitatively and quantitatively analysed by means of high pressure liquid chromatography (HPLC) (P. Maisenbacher et al., Planta Med. 58:351-354 (1992)). The photometric method described in the "Deutsche Arzneimittel-Codex (DAC) [German Drug Codex]" is used for measuring the total hypericins.

Besides the active compound or the active compounds, the ointment or cream according to the invention can comprise various pharmaceutically tolerated cream or ointment bases. Examples of these are white vaseline, viscous paraffin, wool wax, ascorbyl palmitate, glycerol monostearate 60, tocopherol (vitamin E), cetyl alcohol, medium-chain triglycerides, yellow wax, propylene glycol, Macrogol-

1000-glycerol monostearate, citric acid, ascorbic acid and other preservatives and distilled water.

A preferred ointment according to the invention comprises about 15% by weight of St. John's wort extract and white vaseline, viscous paraffin, wool wax and ascorbyl palmitate, in each case in suitable quantity.

A preferred cream according to the invention comprises about 10% by weight of St. John's wort extract and also white vaseline, glycerol monostearate 60, cetyl alcohol, medium-chain triglycerides, yellow wax, propylene glycol, Macrogol-1000-glycerol monostearate, citric acid and water, in each case in suitable quantity.

The present invention also relates to a process for preparing a topical ointment or cream, in which process hyperforin and, where appropriate, hypericins, or a St. John's wort extract which contains at least 200 µg of hyperforin/ml and at least 200 µg of hypericins/ml, is/are mixed with customary pharmaceutically tolerated adjuvants such that an ointment or cream having a minimum content of 15 µg of hyperforin/ml and, where appropriate, a minimum content of 15 µg of hypericins/ml, is obtained.

When a St. John's wort extract is used for preparing the ointment or cream according to the invention, this extract has, in order to protect the hyperforin from oxidation, to be stored in the dark and firmly sealed, and under a protective gas (e.g. argon), until it is processed.

The ointment or cream according to the invention is suitable, for example, for treating cancer diseases, precancerous stages, inflammatory skin diseases, geriatric skin and bacterial skin infections. Because of its fatty base, the ointment is particularly indicated in the case of dry, desquamative skin changes which are accompanied by pruritus or inflammations. The active compound content which is preferred for the ointment (i.e. 15% by weight) is somewhat higher than

the active compound content which is preferred for the cream (i.e. 10% by weight), which latter is, because of its amphiphilic nature, particularly suitable for treating acute to subacute eczematous skin changes.

The ointment according to the invention is consequently particularly suitable for treating chronic and also superinfected eczemas, exsiccation eczemas, hyperkeratotic hand and foot eczemas, subacute to chronic atopic dermatitis (neurodermatitis), lichen simplex, contact eczemas, prurigo simplex subacuta and other prurigo types, and psoriaris vulgaris of the plaque type, and also geriatric skin.

The cream is particularly suitable for treating acute to subacute atopic dermatitis (neurodermatitis), acute to subacute contact eczemas, psoriasis and geriatric skin, and also for the after-treatment and relapse prophylaxis of all eczemas.

The ointment and the cream can also be used in veterinary medicine, for example for treating inflammatory and infected skin diseases, such as mastitis (udder inflammation).

From a concentration of 100 ng/ml and upwards, hyperforin has a proliferation-inhibiting effect on human keratinocytes and lymphocytes. In addition to this, it has been possible to demonstrate that hypericin has a proliferation-inhibiting effect on keratinocytes (HaCaT) and T cells and can induce apoptosis in these cells. This effect is partially mediated by the formation of free oxygen radicals.

Because of the possible photosensitization due to the optional content of hypericin in the ointment or cream according to the invention, investigations were carried out in order to determine whether local use of the hypericin-containing preparation according to the invention can lead to sunburn-like phenomena. These investigations did not show any risk of sunburn.

The ointment or cream according to the invention has the advantage that its base can be adapted to various skin conditions. Thus, the cream is

particularly suitable for treating acute and subacute dermatoses while the ointment is suitable for treating chronic dermatoses. In addition to this, both fat-soluble (hyperforin) and water-soluble (hypericin) active compounds from St. John's wort can be worked into an ointment or cream base. This makes it possible to achieve an effect which is superior to that of the known St. John's wort oil. In addition, the penetration of active compounds from cream and ointment bases is superior to that of active compounds from oils.

The novel ointment or cream for local topical use decisively enriches the spectrum of therapies for inflammatory skin diseases such as neurodermatitis. In particular, the possibility exists of reducing cortisone therapy.

The attached Figure 1 shows the antiproliferative effect of hyperforin on the tumour cell lines HT144 (human melanoma metastasis), A431 (human squamous cell carcinoma), Jurkat (human leukaemic lymphoma), 1F6 (human melanoma, primary tumour) and MT450 (rat mammary carcinoma) (Example 1).

Figure 2 shows the ability of hyperforin to induce apoptosis in the tumour cell lines HT144 (human melanoma metastasis), A431 (human squamous cell carcinoma), Jurkat (human leukaemic lymphoma), 1F6 (human melanoma, primary tumour) and MT450 (rat mammary carcinoma) (Example 2).

Figure 3 shows the apoptosis, due to the selective activation of caspases 9 and 3, which is induced by hyperforin (Example 5).

Figure 4 shows the ability of hyperforin to inhibit the tumour growth of mammary carcinoma cells in vivo in a similar manner to taxol (Example 6).

Figure 5 shows the proliferation-inhibiting effect of the cream according to the invention, as compared with that of St. John's wort oil, following use on healthy test subjects in vivo (Example 9).

Figure 6 shows the proliferation-inhibiting effect of hyperforin on HaCaT cells in vitro (Example 13).

Figure 7 shows the proliferation-inhibiting effect of hyperforin on PBMC in vitro (Example 14).

Figure 8 shows the proliferation-inhibiting effect of a cream which was standardized for hyperforin and of a Hypericum cream as compared with the immunosuppressive effect of a sun simulator irradiation (two times MED). Untreated skin was tested as the control (Example 15).

Figure 9 shows the proliferation-inhibiting effect of hyperforin in vitro (Example 16).

The following examples are intended to clarify the invention. Commercially obtainable hyperforin from HWI Analytik, Rheinzabern, Germany, was used for Examples 1-6. The purity of the hyperforin was greater than 90%. In all the experiments, the solvent DMSO was tested at the maximum concentration used and did not show any effects on proliferation or apoptosis rate.

Example 1

The antiproliferative effect of hyperforin on human and rat tumour cells was investigated in vitro. For this, tumour cells of the tumour cell lines HT144 (human melanoma metastasis), A431 (human squamous cell carcinoma), Jurkat (human leukaemic lymphoma), 1F6 (human melanoma, primary tumour) and MT450 (rat mammary carcinoma) were cultured, at a concentration of 1×10^5 cells/ml, in 1640 RPMI containing 10% foetal calf serum (FCS) containing 1% penicillin/streptomycin (all from Gibco) in 96-well microtitre plates (37°C, 5% CO_2). Hyperforin (HWI-Analytik) which had been freshly dissolved in DMSO was added, at various concentrations, to these cells for 24 h. 1 µCurie of ^{3}H-thymidine (Amersham) was then added per well, and the incorporated radioactivity was measured in a scintillation counter (Canberra Packard) after 18 h.

The radioactivity which is measured is proportional to the replication of the DNA in the cells.

It can be seen from Figure 1 that the concentration of hyperforin at which the growth of the cells was inhibited by 50% (IC_{50}) was between 5 and 15 µm.

Example 2

This example proves that hyperforin induces apoptosis, i.e. what is termed programmed cell death, in tumour cells. The induction of apoptosis in tumour cells is a characteristic feature of many cytostatic agents and provides supports for the view that hyperforin acts as such an agent.

HT144 (human melanoma metastasis), A431 (human squamous cell carcinoma), Jurkat (human leukaemic lymphoma), 1F6 (human melanoma, primary tumour) and MT450 (rat mammary carcinoma) tumour cells were cultured, at a concentration of 1×10^4 cells/ml, in 96-well microtitre plates. After the cells had been preincubated for 24 h, hyperforin was pipetted in to give the final concentrations shown in Figure 2. The cells were then lysed and then examined for low molecular weight DNA fragments using a Cell Death Detection ELISA^{PLUS} (Boehringer, Mannheim). For this, use was made of a biotinylated anti-histon antibody and a peroxidase-coupled anti-DNA antibody, and the proportion of low molecular weight DNA was determined by measuring the absorption of peroxidase at 405 nm.

The results are shown in Figure 2, which depicts the extinction at 405 nm after subtracting the untreated control. It can be seen that hyperforin induces apoptosis, in a dose-dependent manner, in all the tumour cell lines.

Example 3

The toxic effect of various concentrations of hyperforin on the tumour cell lines HT144, A431 and Jurkat used in Examples 1 and 2 was investigated using

a cytotoxicity assay. For this, membrane integrity was determined by means of trypan blue exclusion. The result is shown in Table 1, with the values being given in % of the untreated cells. It was scarcely possible to demonstrate any toxic effects, thereby confirming that hyperforin induces apoptosis specifically.

Table 1

Hyperforin (µg/ml)	Trypan blue exclusion (% of the cells)		
	HT144	A431	Jurkat
0	100	100	100
2.5	100	100	100
5	100	100	100
10	100	100	100
20	100	95	100
40	90	90	100
80	80	90	100

Example 4

The following example proves that hyperforin and the known cytostatic agent taxol, used as a comparison substance, induce apoptotic DNA fragmentation. The DNA fragmentation was determined by means of DNA gel electrophoresis. Jurkat (leukaemia) was used as the tumour cell line.

In each case 1 x 10^6 cells were incubated at 37°C while being untreated or treated with hyperforin (40 µM) or taxol (10 µM), respectively. Apoptotic DNA fragments were isolated by means of lysis with NP 40. The cells were washed and pelleted after 4 hours or after 24 hours, respectively. The cell pellet was incubated for 10 sec. with lysis buffer (1% NP 40,

Sigma; 20 mM EDTA, Sigma; 50 mM Tris-HCl, Sigma). The lysates were mixed with 1% SDS (Sigma), incubated at 56°C for 2 h with RNase (5 µg/µl) (Boehringer), and digested with proteinase K (Sigma) (2.5 µg/µl) for 2 h at 37°C. After 10 M ammonium acetate had been added, the DNA was precipitated with 100% ethanol at -20°C and analysed by means of gel electrophoresis on 1% agarose gels.

The results are shown in Table 2. It was found that hyperforin induces apoptosis in tumour cells more rapidly than does taxol, since the apoptosis was fully developed after only 4 hours.

Table 2

	Untreated cells	Hyperforin (40 µM)	Taxol (10 µM)
4 hours	-	++	+
24 hours	-	++	++

++ = strongly positive
\+ = positive
\- = negative

Example 5

In order to demonstrate a possible mechanism of action for the induction of apoptosis by hyperforin, the activities of different caspases in tumour cell lines were investigated. The activation of caspases can be effected by a variety of signal transduction mechanisms and leads, by way of the activation of effector caspases (e.g. caspase 3) to induction of the programmed cell death. The activities of upstream caspase 9, downstream caspase 8 and effector caspase 3

were measured using a commercially available caspase kit (R&D Systems). MT450 cells were incubated with or without hyperforin (final concentration 50 µM) for 24 h at a concentration of 1 million cells/ml. After that, the cells were centrifuged down and the supernatant was removed; the cells were then lysed with lysis buffer. The cell lysate was in each case incubated with a substrate which was specific for the caspase and the cleavage product, which was coupled to a dye, was detected photometrically in an ELISA reader.

The effect on caspase activity relative to the untreated control (control = 1) is shown in Figure 3. It is found that while hyperforin leads to an upregulation of caspase 9 and caspase 3, it does not lead to any activation of caspase 8.

Example 6

The effect of hyperforin on the growth of MT450 cells (mammary carcinoma) was investigated in vivo in rats. A treatment with the same concentrations of the cytostatic agent taxol was carried out for comparison. Each treatment group consisted of 8 experimental animals; the treatment was carried out blind (blind experiment). 1 million tumour cells were injected into each animal. Intratumour injections were started after 3 weeks. The injections were performed daily, using in each case 100 µl of the solvent (10% DMSO in PBS), of the hyperforin (500 µg in 10% DMSO/PBS) or of the taxol (500 µg in 10% DMSO/PBS). The tumour volume was measured planimetrically 8 times, from the start of the tumour injections (day 0) until the conclusion of the treatment (day 30) and recorded as a growth curve (mean value +/- standard deviation) in Figure 4. It is found that hyperforin significantly inhibits the growth of MT405 cells in vivo to the same extent as does taxol.

Example 7

A St. John's wort ointment was prepared in accordance with the following recipe (values in % by weight):

White Vaseline	50.0
Viscous paraffin	9.0
Wool wax	25.0
Ascorbyl palmitate	1.0
St. John's wort extract	15.0
	100

The Vaseline, the viscous paraffin and the wool wax were heated to 60°C in a water bath and mixed. The mixture was allowed to cool, while being stirred, with the ascorbyl palmitate being worked in during this period; the St. John's wort extract (total extract supplied by Caelo (hyperforin 240 µg/ml, hypericin 300 µg/ml)) was then worked in after the mixture had cooled down. The ointment which thus resulted contained 36 µg of hyperforin/ml and 45 µg of hypericin/ml.

Example 8

A St. John's wort cream was prepared in accordance with the following recipe (values in % by weight):

White Vaseline	20.0
Glycerol monostearate 60	4.0
Cetyl alcohol	6.0
Medium-chain triglycerides	8.0
Yellow wax	4.0
Propylene glycol	10.0
Macrogol-1000-glycerol monostearate	7.0
Citric acid	1.0
Distilled water	30.0
St. John's wort extract	10.0
	100

The vaseline, the glycerol monostearate 60, the cetyl alcohol, the medium-chain triglycerides and the yellow wax were heated to 60°C in a water bath and mixed. The Macrogol-1000-glycerol monostearate, the propylene glycol, the water and the citric acid were heated separately to 60°C in a water bath and then worked into the first mixture. The resulting mixture was stirred until it had cooled down and the St. John's wort extract (total extract supplied by Caelo (hyperforin 240 µg/ml, hypericin 300 µg/ml)) was then worked in. The cream which thus resulted contained 24 µg of hyperforin/ml and 30 µg of hypericin/ml.

Example 9

An extract supplied by Flavix (Rehlingen), which had a hyperforin content of 20% by weight (20 g/100 g) and to which neutral oil had been added, was used to prepare a hyperforin ointment or cream which contained a higher concentration of active compound. For this, 5 g of the extract were dissolved in 10 ml of 70% ethanol in order to obtain a starting solution having a hyperforin concentration of 100 mg/ml. This starting solution was worked into an ointment or cream base, in the manner described in Example 7 and Example 8, respectively, in place of the St. John's wort extract supplied by Caelo. Ointments and creams were prepared using the following recipes (values in % by weight):

a) Ointment

	1% hyperforin	**0.1% hyperforin**
White Vaseline	50.0	50.0
Viscous paraffin	9.0	9.0
Wool wax	30.0	39.0
Ascorbyl palmitate	1.0	1.0
Starting solution	10.0	1.0
	100.0	100.0

b) Cream

	1% hyperforin	**0.1% hyperforin**
White Vaseline	20.0	20.0
Glycerol monostearate	4.0	4.0
Cetyl alcohol	6.0	6.0
Medium-chain triglycerides	8.0	17.0
Yellow wax	4.0	4.0
Propylene glycol	10.0	10.0
Macrogol-1000-glycerol monostearate	7.0	7.0
Citric acid	1.0	1.0
Distilled water	30.0	30.0
Starting solution	10.0	1.0
	100.0	100.0

Example 10

The immunomodulatory effects of the novel preparation on the skin were examined in humans (3x4 test subjects) ex vivo after using the St. John's wort ointment from Example 7 in comparison with St. John's wort oil. For this, skin samples, which had been subjected to different treatments, were removed from voluntary test subjects and an investigation was carried out in order to determine whether the ability of epidermal Langerhans cells to present antigen is being affected.

In detail, the following MECLR (mixed epidermal cell leukocyte reaction) was carried out: In 4 test subjects in each case, circular test areas of 2 cm in diameter on the flexor sides of the forearm were treated either with St. John's wort oil, St. John's wort cream or the treatment base, and the effect was examined in comparison with untreated skin. 100 µl of the test substances were applied for 24 h in epicutaneous test chambers. After that, the residues were removed and epidermal suction blisters were

produced using a vacuum. The roof of the blister was dissected out under sterile conditions using a scalpel and a suspension of epidermal cells (EC) was prepared by treating with trypsin. 50,000 EC were cocultured for 6 days (37°C, 5% CO_2) with 150,000 T cells (TC) in RPMI 1640 containing 10% foetal calf serum (FCS) containing 1% penicillin/streptomycin (all from Gibco) in 96-well flat-bottomed microtitre plates (Greiner). 1 µCurie of ^{3}H-thymidine (Amersham) was then added per well and the radioactivity which was incorporated was measured in a scintillation counter (Canberra Packard). The radioactivity which is measured is proportional to the replication of the DNA in the cells.

These investigations showed that the St. John's wort ointment according to the invention brings about an inhibition of proliferation. On the other hand, the use of St. John's wort oil results in an increase in proliferation (Figure 5).

These results provide support for the St. John's wort ointment according to the invention having an antiinflammatory effect which was not detectable in the case of the oil.

Example 11

In a unilateral experiment carried out on a patient suffering from eczema, one lower leg was treated for two weeks with St. John's wort oil while the other was treated with the novel St. John's wort ointment as described in Example 7. At the time of inspection, the lower leg which had been treated with the ointment had recovered very well while the lower leg which had been treated with the oil had, if anything, deteriorated.

Example 12

Four patients suffering from different forms of localized eczema were treated. After the experiment had been explained and the patients had consented, the eczemas were documented photographically and the

patients carried out a monotherapy with the ointment according to the invention over a period of 2 weeks. After that, the eczemas were documented photographically once again. Complete healing was achieved in two of the patients while the condition was substantially improved in the other patients. The results of this treatment are summarized in Table 3.

Table 3

No.	Sex	Diagnosis	Treatment	Success of the therapy
1	female	Eczema in the hollow of the elbow or knee	Hypericum cream	healed
2	male	Eczema of the hand	Hypericum cream	improved
3	female	Neurodermatitis of the arms	Hypericum cream	healed
4	female	Prurigo of the lower leg	Hypericum cream	improved

Example 13

This example demonstrates the proliferation-inhibiting effect of hyperforin on keratinocytes. HaCaT cells were cultured (37°C, 5% CO_2) in keratinocyte medium containing 10% foetal calf serum (FCS) containing 1% penicillin/streptomycin (all from Gibco). Subconfluent cultures were detached using EDTA-trypsin (Gibco), washed 3x in PBS and then cultured for a further 24 h (until adherence) at a density of 20,000/well in 96-well flat-bottomed microtitre plates (Greiner). After that, hyperforin (HWI-Analytik) which had been freshly dissolved in DMSO was added for a period of 24 h. 1 µCurie of ^{3}H-thymidine (Amersham) was then added per well and the incorporated radioactivity was measured in a scintillation counter (Canberra Packard). The radioactivity which is measured is proportional to the replication of the DNA in the

cells. The results for hyperforin concentrations of from 0 to 100 µg/ml are depicted in Figure 6, where cpm denotes counts per minute. It is found that proliferation is virtually completely inhibited at a hyperforin concentration of 100 µg/ml.

Example 14

This example demonstrates the proliferation-inhibiting effect of hyperforin on peripheral blood mononuclear cells (PBMC). PBMC were isolated from heparinized blood by density gradient centrifugation using Ficoll (Seromed). The PBMC were washed 3x in PBS and cultured for 24 h (37°C, 5% CO_2) in RPMI 1640 containing 10% foetal calf serum (FCS) containing 1% penicillin/streptomycin (all from Gibco) in 96-well flat-bottomed microtitre plates (Greiner) at a density of 200,000/well. After that, the cells were stimulated with 1 µg/ml phytohaemagglutinin (PHA) (Wellcome), and hyperforin (HWI-Analytik) which had been freshly dissolved in DMSO was added for a period of 24 h. 1 µCurie of ^{3}H-thymidine (Amersham) was then added per well and the incorporated radioactivity was measured in a scintillation counter (Canberra Packard). The radioactivity which is measured is proportional to the replication of the DNA in the cells. The results for hyperforin concentrations of from 0 to 100 µg/ml are depicted in Figure 7, where cpm denotes counts per minute. It is found that even small concentrations of hyperforin have a proliferation-inhibiting effect on PBMC and proliferation is virtually completely inhibited at a concentration of 100 µg/ml.

Example 15

This example shows that hyperforin cream containing a high proportion of hyperforin has an immunomodulatory effect in vivo. It was possible to demonstrate, by means of experiments using purified hyperforin, that this immunomodulatory effect is to be attributed to the hyperforin.

The immunomodulatory effect of the cream according to the invention was tested ex vivo in humans (in each case 4 test subjects). For this, skin samples which had been treated in different ways were removed and an investigation was carried out to determine whether the ability of epidermal Langerhans cells to present antigen is affected. In detail, this was investigated in an MECLR (mixed epidermal cell leukocyte reaction): In 4 test subjects in each case, round test areas of 2 cm in diameter on the flexor side of the lower arm were treated with Hypericum cream (containing 24 µg of hyperforin/ml and 30 µg of hyperin/ml), with hyperforin cream (24 µg/ml) or with sun simulator irradiation (144 J/cm^2). Untreated skin and the use of the vehicle without active compounds served as the controls. 100 µl of the test substances were applied for 24 h in epicutaneous test chambers. After that, the residues were removed and an epidermal suction blister was produced using a vacuum. The roof of the blister was dissected out under sterile conditions using a scalpel and a suspension of epidermal cells (EC) was prepared by treating with trypsin. 50,000 EC were cocultured for 6 days (37°C, 5% CO_2) with 150,000 TC (T cells) in 1640 RPMI containing 10% foetal calf serum (FCS) containing 1% penicillin-streptomycin in 96-well flat-bottomed microtitre plates (Greiner). 1 µCurie of ^{3}H-thymidine (Amersham) was then added per well and, after 18 h, the incorporated radioactivity was measured in a scintillation counter (Canberra Packard). The radioactivity which is measured is proportional to the replication of the DNA in the cells.

The results (Figure 8) show that the hyperforin-containing St. John's wort cream and the hyperforin cream significantly inhibit proliferation to the same order of magnitude as does irradiation with the sun simulator.

Example 16

This example demonstrates the proliferation-inhibiting effect of hyperforin in vitro. In all cases, use was made of pure hyperforin from HWI-Analytik (Rheinzabern). The purity of the hyperforin was > 90%. In all the in-vitro experiments, the solvent DMSO was tested at the maximum concentration employed and did not exhibit any effect on the proliferation and vitality of the cells. Normal skin samples were removed from healthy test subjects; these samples were then incubated with hyperforin in vitro and an investigation was carried out to determine whether the ability of epidermal Langerhans cells to present antigen is affected.

This was investigated in an MECLR (mixed epidermal cell leukocyte reaction) as described in Example 14: In 4 test subjects in each case, epidermal suction blisters were produced on the flexor side of the lower arm using a vacuum. The roof of the blister was dissected out under sterile conditions using a scalpel and a suspension of epidermal cells (EC) was prepared by treating with trypsin. A part of the EC or the TC was in each case incubated for 24 h with 24 µg of hyperforin/ml. After that, the cells were washed and 50,000 EC were cocultured for 6 days (37°C, 5% CO_2) with 150,000 TC (T cells) in 1640 RPMI containing 10% foetal calf serum (FCS) containing 1% penicillin-streptomycin in 96-well flat-bottomed microtitre plates (Greiner). 1 µCurie of ^{3}H-thymidine (Amersham) was then added per well and, after 18 h, the incorporated radioactivity was measured in a scintillation counter (Canberra Packard). The radioactivity which is measured is proportional to the replication of the DNA in the cells.

The results (Figure 9) show that hyperforin significantly inhibits proliferation both when acting on EC and when acting on TC.

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Patent Claims

1. Use of hyperforin for producing a drug for treating cancer diseases and/or precancerous stages.

2. Use according to Claim 1 for treating lymphomas and/or leukaemias.

3. Use according to Claim 1 for treating metastases, in particular melanoma metastases.

4. Use according to Claim 1 for treating epithelial tumours and/or epithelial precancerous stages.

5. Topical ointment or cream which comprises at least 15 µg of hyperforin/ml.

6. Ointment or cream according to Claim 5 which comprises 0.02-20 mg of hyperforin/ml, preferably 1-20 mg of hyperforin/ml.

7. Ointment or cream according to one of Claims 5 and 6 which additionally comprises at least 15 µg of hypericins/ml.

8. Ointment or cream according to Claim 7 which comprises at least 5% by weight of St. John's wort extract which contains at least 200 µg of hyperforin/ml and at least 200 µg of hypericins/ml.

9. Ointment or cream according to Claim 8 in which the St. John's wort extract contains 200-100,000 µg/ml, preferably about 1000 µg of hyperforin/ml and 200-2000 µg of hypericins/ml.

10. Ointment or cream according to Claim 8 or 9 in which the St. John's wort extract contains 20-60% v/v of ethanol.

11. Ointment according to one of Claims 7-10 which comprises about 15% by weight of St. John's wort extract and also a customary ointment base.

12. Cream according to one of Claims 7-10 which comprises about 10% by weight of St. John's wort extract and also a customary cream base.

13. Process for producing a topical ointment or cream according to one of Claims 5-12, in which process hyperforin and, where appropriate, hypericins, or a St. John's wort extract which contains at least 200 µg of hyperforin/ml and at least 200 µg of hypericins/ml, is/are mixed with customary pharmaceutically tolerated adjuvants such that an ointment or cream having a minimum content of 15 µg of hyperforin/ml, preferably 1-20 mg of hyperforin/ml, and, where appropriate, 15 µg of hypericins/ml, is obtained.

14. Use of an ointment or cream according to one of Claims 5-12 for treating cancer diseases, precancerous stages, inflammatory skin diseases, geriatric skin or bacterial skin infections.

15. Use according to Claim 14 in which an ointment according to one of Claims 5-11 is used for treating chronic, and also superinfected, eczemas, exsiccation eczemas, hyperkeratotic hand and foot eczemas, a subacute to chronic atopic dermatitis (neurodermatitis), lichen simplex, contact eczemas, prurigo simplex subacuta and other prurigo types and psoriaris vulgaris of the plaque type and also geriatric skin.

16. Use according to Claim 14 in which a cream according to one of Claims 5-10 or 12 is used for treating an acute to subacute atopic dermatitis (neurodermatitis), an acute to subacute contact eczema, psoriasis guttata or geriatric skin, or for the after-treatment and relapse prophylaxis of all eczemas.

17. Use according to Claim 14 for treating inflammatory superinfected skin diseases in veterinary medicine.

18. Process for treating cancer diseases and/or precancerous stages by administering hyperforin.

19. Process for treating cancer diseases, precancerous stages, inflammatory skin diseases, geriatric skin or bacterial skin infections by applying an ointment or cream according to one of Claims 5-12 to the skin.

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Abstract

Hyperforin as a cytostatic agent, and hyperforin ointment or cream as an application form

The invention relates to the use of hyperforin for treating cancer diseases and/or precancerous stages. In addition, the invention relates to a hyperforin-containing ointment or cream and to its production and use. The hyperforin-containing ointment or cream is also suitable for treating inflammatory skin diseases, geriatric skin and bacterial skin diseases and also skin diseases in the sphere of veterinary medicine.

Jan SIMON *et al*

HYPERFORIN AS A CYTOSTATIC AGENT AND HYPERFORIN OINTMENT OR CREAM AS AN APPLICATION FORM

24741-1525

Figure 1

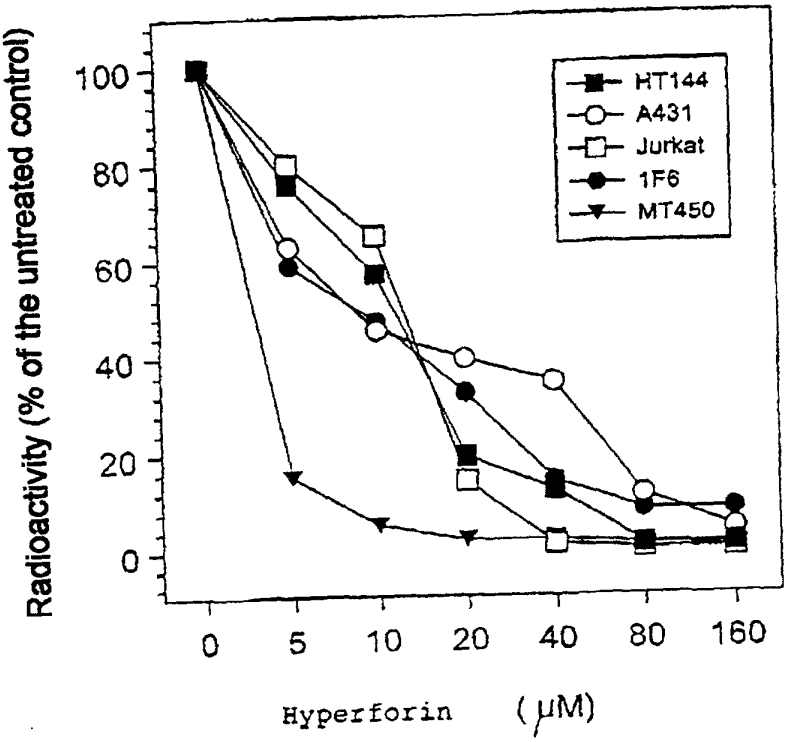

Figure 2

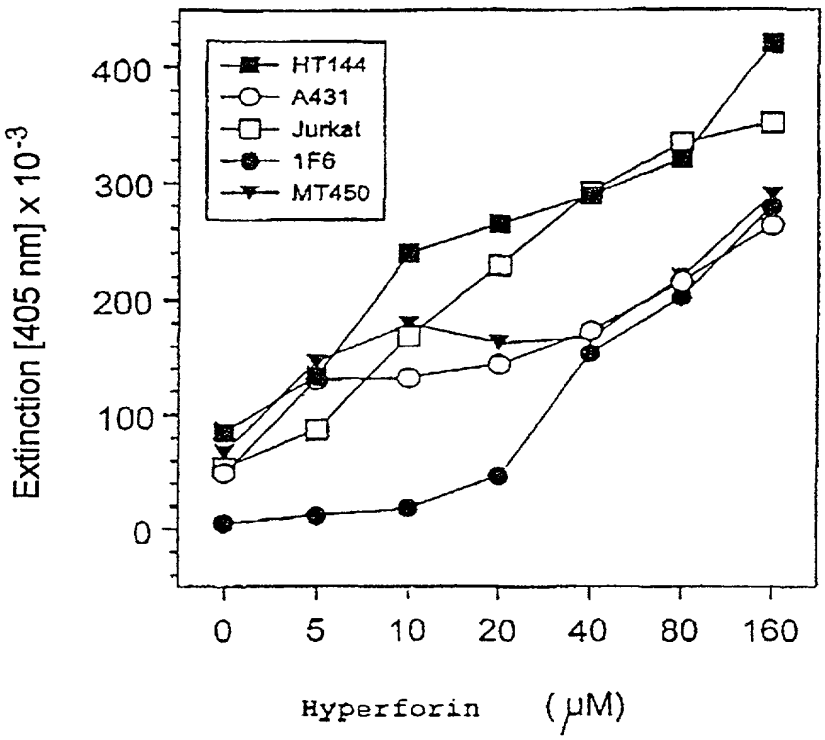

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Figure 3

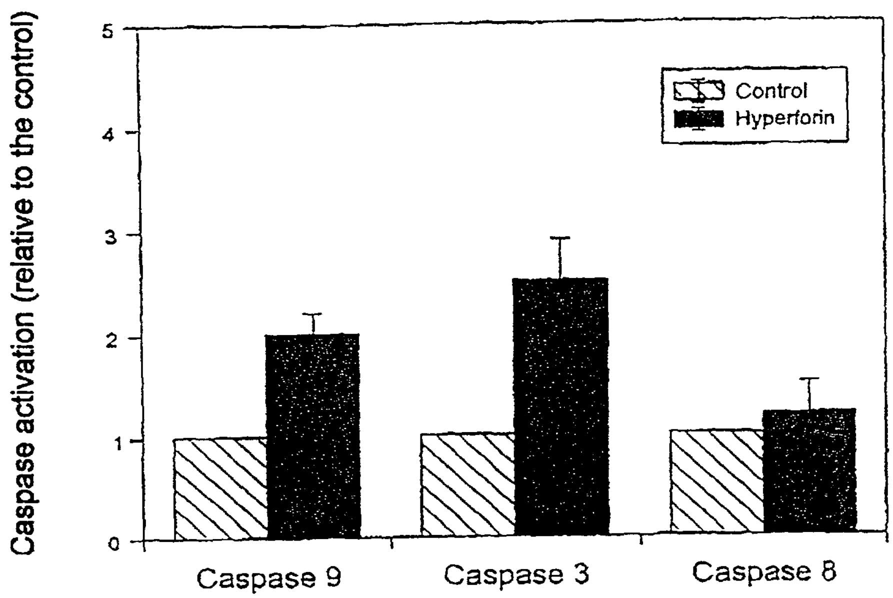

Figure 4

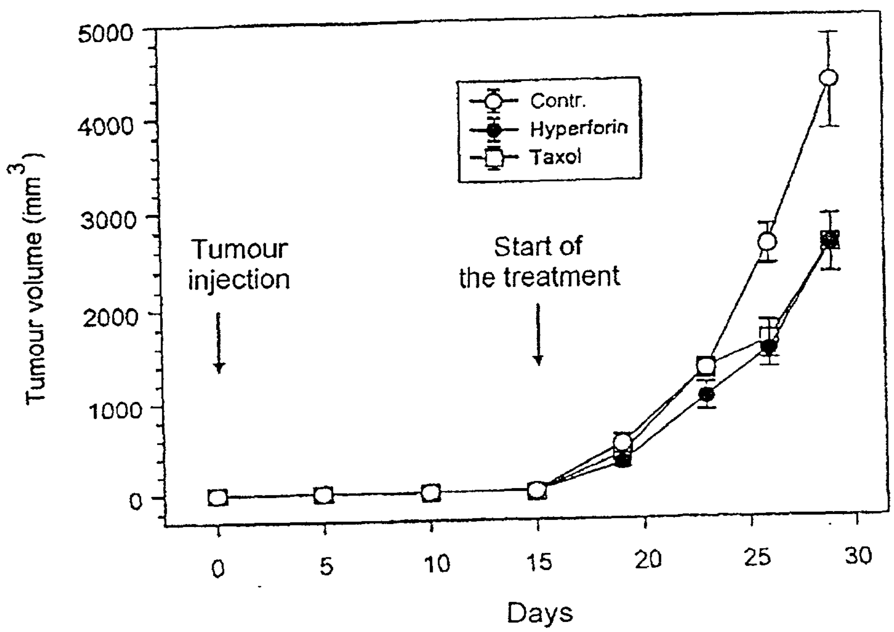

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Figure 5

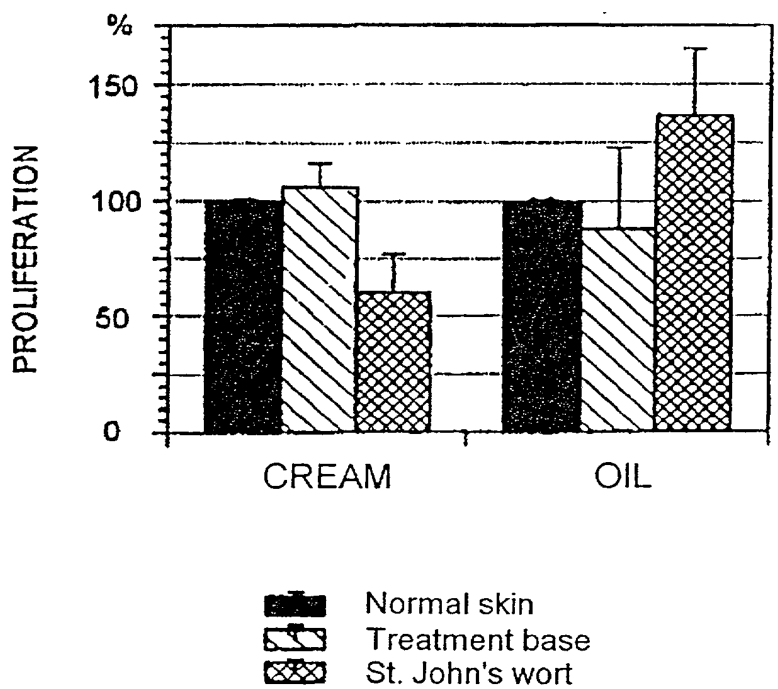


Figure 6

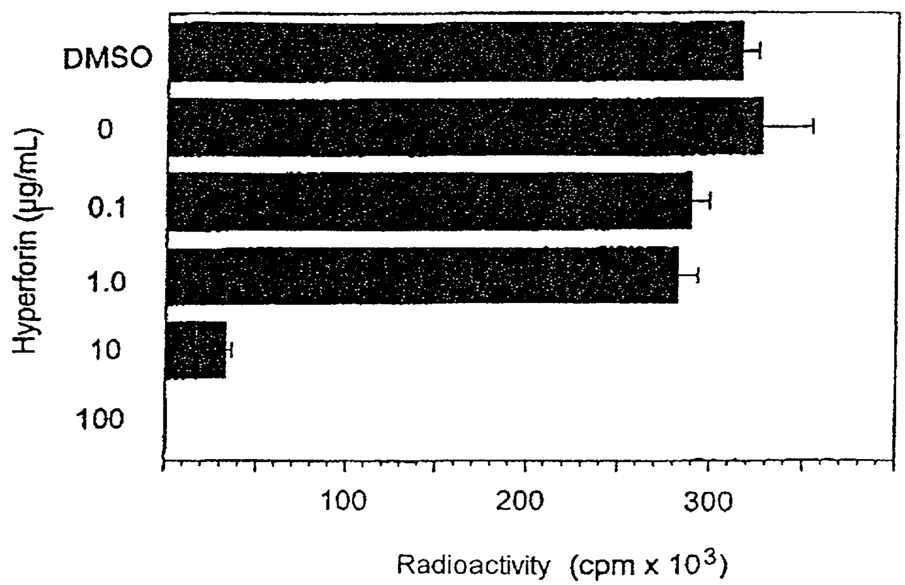


Figure 7

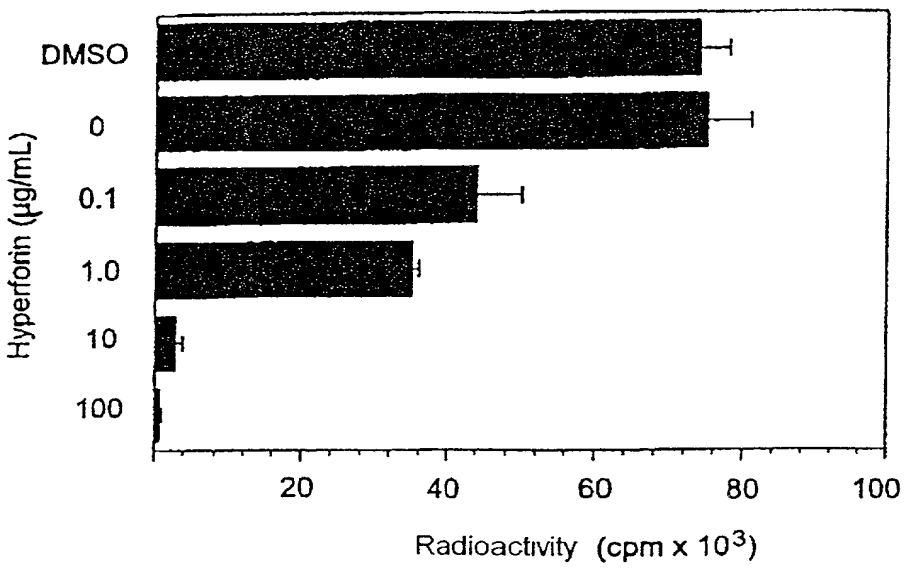


Figure 8

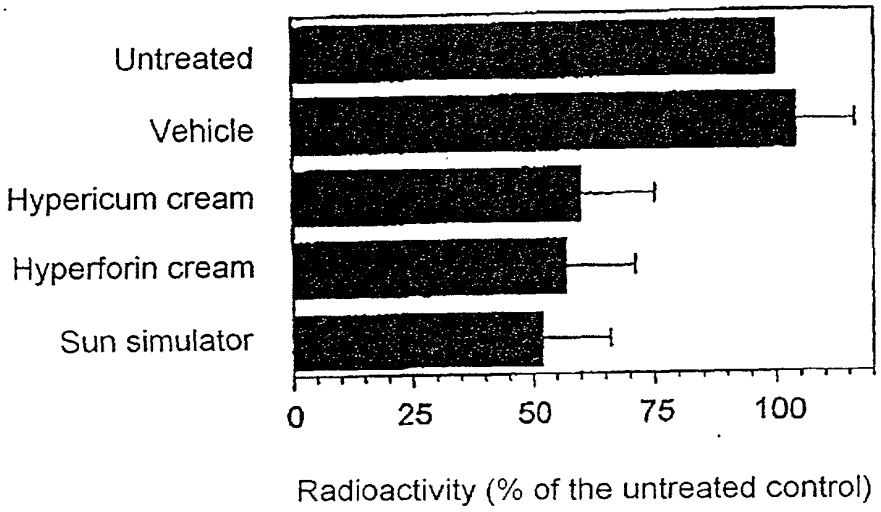
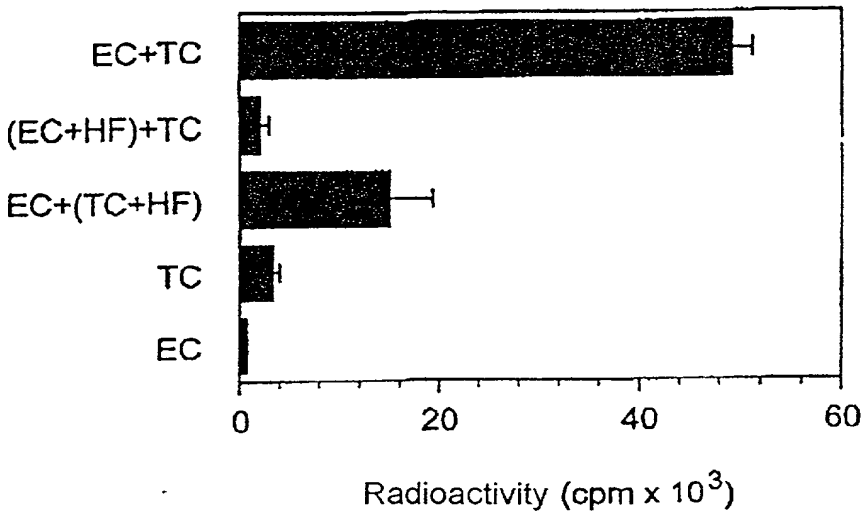


Figure 9



Attorney Docket No.: 24741-1525

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

HYPERFORIN AS A CYTOSTATIC AGENT AND HYPERFORIN OINTMENT OR CREAM AS AN APPLICATION FORM

the specification of which (check one)

___ is attached hereto.

__X__ was filed on **November 24, 1999** as PCT International Application Number **PCT/EP99/09067** and was amended on

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

THAT I do not know and do not believe that the same invention was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code §119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's

certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number	Country	Foreign Filing Date	Priority Claimed?	Certified Copy Attached?
198 54 446.4	**Germany**	**November 25, 1998**	**Yes**	**No**
199 13 333.6	**Germany**	**March 24, 1999**	**Yes**	**No**

I HEREBY CLAIM the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date

I HEREBY CLAIM the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number

I HEREBY APPOINT the following registered attorneys and agents of the law firm of **Heller Ehrman White & McAuliffe** to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith:

6

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I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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